er, most of the patients in the hemolysis study were less than 13 days old, and the protocol required blood collection for the study be from one skin puncture—both factors known to increase the incidence of hemolysis in blood collection by skin puncture (3). Samples from patients older than 13 days, in whom blood was easily obtained from one skin puncture, had a 5% incidence of semiquantitative hemolysis for both types of tubes. Plasma yield averaged 51% with each container.

Handling time for samples obtained with Microtainer Tubes was significantly reduced as a result of (a) the avoidance of the scoring and breaking procedure required for Caraway-tube specimens; (b) the larger volume of the Microtainer Tube (equivalent to two Caraway tubes), which eliminated handling of extra tubes on many samples; and (c) the short centrifugation time in the Microfuge to separate plasma from cells in the Microtainer Tubes [a brief study showed that normal specimens in Microtainer tubes required as little as 1 min of centrifugation time for effective separation (data not shown)].

In summary, we found Microtainer Tubes to be generally as effective as Caraway tubes in providing accurate chemistry results, in allowing recovery of plasma, and in preserving the cellular integrity of the samples. Many laboratories may find the savings in handling time and the decreased possibility of blood-borne infection with Microtainer Tubes to be persuasive factors in selecting a capillary blood-collecting container.

References


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Use of Magnetic Circular Dichroism in the Diagnosis of Porphyria

To the Editor:

Detection and accurate diagnosis of the porphyrias continue to be bedeviled by confusion and controversy for many reasons, including their comparative rarity and their variability in both clinical and biochemical expression. Qualitative and semiquantitative screening methods provide time-saving support for the more nearly accurate and informative porphyrin and enzyme assays (1). Most current screening methods, which have been developed to investigate urines only (2-4), are inadequate if, as is usually the case, they are used alone. Failure to screen fecal porphyrins may have serious, even fatal, consequences (5). Thus the screening of fecal porphyrins is essential, especially for detection of the large but as-yet-unknown proportion of asymptomatic cases. A recent paper on this subject (6) contains serious flaws and, in my opinion, merely adds to the confusion. Although I collaborated in the research project, I withdrew as co-author of the article because of disagreements with the draft submitted to Clinical Chemistry, which I wish to discuss here.

Some of the flaws are common to both the urinary and fecal data, but I shall concentrate on the latter because the fecal screening method, if valid, would represent the most innovative aspect of the article.

Firstly, no standard curves have been included for mesoporphyrin and deuteroporphyrin, both of which frequently constitute substantial fractions of normal fecal porphyrins, or for the isocoproporphyrin fractions, which often predominate in the feces of subjects with porphyria cutanea tarda, arguably the most common of the porphyrias (1). With this in mind, the claim that the technique involving MCD [magnetic circular dichroism] is capable of identifying the major porphyrin in the urine or feces is unsubstantiated, because λ_{max} and λ_{min} values for these other porphyrins are lacking, and any shift in these wavelengths produced by the various mixtures of porphyrins commonly found in normal and porphyrin excreta has not been measured or adequately discussed.

It was stated that Figure 5 shows that about 65% of the porphyrins are extracted from Dean's solution... into HCl... . The word "about" may allow for the substantial errors to be expected when the extraction procedure described is used, a procedure not developed for quantitative purposes, yet neither error bars nor any hard data are provided in the Figure (as also is the case in Figure 4) and no standard deviations are provided in the text. The "Procedures" section indicates that the standards were made up by adding porphyrins to fecal samples before extraction, a poor substitute for measuring the efficiency with which Dean's solution extracts the porphyrins that are naturally present in feces. In my experience and that of others (5), this may vary markedly, depending mainly on the water, lipid, total porphyrin, and bacterial content of the particular fecal sample. Furthermore, it is not explained how (or whether) the original porphyrin content of the excreta to which the standard porphyrins were added was either measured or allowed for. Without full insight into the substantial errors involved in the standard curves, it is questionable that they can be used for even semiquantitative measurements, especially for diagnostic purposes. Yet this is what the authors recommend, albeit with the suggestion that the acceptable upper limit of the normal range should be reduced by 25% to "<150 nmol/g dry weight of feces." The inadequacy of this
suggestion may be judged by reference to their Table 1 in which the instrumental data, calculated from their recommended standard curve, varies from the reference thin-layer chromatographic (TLC) data by more than ±25% in 66% of their cases, and by more than ±50% in 33% of their cases. In their regression analyses of the urinary and fecal data the authors have not provided (7) standard errors of estimate or the standard deviations of the slopes and intercepts. Because errors are present in the MCD data and, to a lesser extent, in the reference TLC data, purists might argue that regression analysis cannot be performed in this study. Nevertheless, when a new method is compared with a reference method, the reference data are generally used as the regressors ("x values"), yet the authors have used their MCD data from their Table 1 as regressors (I below). When the data are replotted with the reference TLC data as the regressors, quite different results are obtained, as shown in II in the following tabulation. The correlation coefficient, of course, remains the same, 0.588. Significance tests should have been provided for the values given at a chosen critical p value. If the data are not normally distributed—and they appear to be skewed—then the method and significance tests may well be invalidated. The question of whether these data are normally distributed or skewed could only be answered satisfactorily by sampling a much larger number of subjects more nearly covering the expected range of fecal porphyrin values.

A further, and possibly the most suspect, aspect of the statistical approach is the crucial recommendation that for the standard curve, standards of protoporphyrin to coproporphyrin in equimolar ratio should be used. Elsewhere in the article the average molar ratio of these two porphyrins in feces was found to be 8:1. No basis is offered for use of the 1:1 ratio other than that the "standard curve gave far better agreement with the TLC results." This adjustment of the data to improve the statistics is scientifically unacceptable and even further invalidates the proposed procedure for screening fecal porphyrins.

Lastly, the fecal data are given in units of nmol/g dry weight. Even though no mention is made in the "Procedures" section, portions of each fecal sample investigated should have been homogenized, weighed, thoroughly dried, and reweighed—procedures that take several hours and invalidate the authors' basic claim that the method is "rapid, reliable, and involving little sample preparation."

I am not implying that MCD cannot be used for semiquantitative screening of porphyrins in urine or even in feces. Although its use for feces cannot be "rapid" with "little sample preparation," it may have advantages related to the fourfold or greater differences in molar ellipticities of protoporphyrin when compared with coproporphyrin or the acetate-substituted porphyrins (see Figures 4 and 5 in 6). This implies that the technique is markedly more sensitive to all porphyrins other than protoporphyrin and (probably) the other dicarboxylic porphyrins. In normal subjects, fecal protoporphyrin and total porphyrin concentration are closely related (8). In contrast, all the porphyrins except erythropoietic protoporphyrin are characterized, to some degree, by increased concentrations in feces of various combinations of coproporphyrin and (or) the acetate-substituted porphyrins, for which MCD is at least fourfold more sensitive. Therefore, the optimal MCD standard curve should be based on the above-mentioned molar ratio of protoporphyrin to coproporphyrin found in normal feces, >8:1, and the reference interval should only be set when all errors in the technique have been evaluated. Abnormally high amounts of any porphyrin other than protoporphyrin in feces would produce an exaggerated MCD result, which may explain the skewed MCD (8:1) data in the authors' Table 1. However, such circumstances are extremely rare in conditions other than the porphyrinas themselves, indicating that well-evaluated MCD might provide an unusually sensitive screening method for detecting the porphyrinas. Verification might be provided by calculating the correct MCD result for each individual porphyrin present in fecal samples from porphyrin subjects, investigating sufficient numbers of subjects with each type of porphyrin, and comparing data for each porphyrin group with data for many normal subjects and subjects with non-porphyrinic conditions.

References
4. Jones KG, Sweeney GD. Quantitation of urinary porphyrins by use of second deriva-


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The authors of the paper in question respond to the points raised by Dr. Day:

To the Editor:

Rapid qualitative screening of urinary porphyrin is accepted to be highly useful in diagnosis of the porphyrinas (1).

Deuteroporphyrin plus mesoporphyrin appear to constitute an extremely small proportion (<5%) of fecal porphyrin in normal subjects (2). Standard curves for deuteroporphyrin and mesoporphyrin are linear to above 1500 nmol/L and exhibit peak-to-trough intensities in MCD of 5.3 and 4.8 m² cm⁻¹ per nmol/L. Deuter- and mesoporphyrin are characterized by λ_max of 402 and 406 nm and λ_min of 396 and 397 nm, respectively, in MCD. The intensities are similar to that of coproporphyrin.

Coproporphyrin, which is only found in excess in some cases of porphyrin cutanea tarda, would be expected to exhibit peak-to-trough intensities similar to coproporphyrin.

MCD is capable of distinguishing between uro-, copro-, and protoporphyrin when one of these predominates in a mixture of porphyrins, as is normally the case in urine and feces.

"About 65%" refers to the average of 70 ± 4% and 63 ± 4% efficiency of extraction for coproporphyrin and protoporphyrin, not to substantial errors in the extraction of porphyrins.

Day's proposal is an extremely novel one to do a standard curve, and not one we considered.

The porphyrin content of fecal samples utilized for standard curves was <5 nmol. The porphyrin content of the feces was corrected for in standard curves, as evidenced by standard curves passing through the origin.

The underlying assumption that